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Title: Effects of Calcium Ascorbate and Ionizing Radiation on the Survival of *Listeria monocytogenes* and Product Quality of Fresh-cut "Gala" Apples

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Effects of Calcium Ascorbate and Ionizing Radiation on the Survival of *Listeria monocytogenes* and Product Quality of Fresh-cut 'Gala' Apples

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ABSTRACT: The interactive effects of calcium ascorbate (CaA) and ionizing radiation on viability of *Listeria monocytogenes* inoculated in solutions and on 'Gala' apple slices were investigated. The D_{10} values (radiation doses that inactivate 90% of bacterial population) for *L. monocytogenes* inoculated in water, 3.5%, and 7.0% CaA solutions were 0.32, 0.61, and 0.58 kGy, respectively. The D_{10} values of the pathogen on the surface of apple slices treated with water, 3.5%, and 7.0% CaA were 0.24, 0.32, and 0.32 kGy, respectively. To determine the impact of CaA and irradiation on quality of apple slices, apple slices treated with 0%, 3.5%, and 7.0% CaA were exposed to 1.6 kGy gamma radiation (a dose that produced a 5-log reduction of *L. monocytogenes*) and stored under modified atmosphere at 4 °C for 14 d. CaA at levels of both 3.5% and 7.0% prevented the browning of the apple slices. The apple aroma intensity, however, decreased as the concentration of CaA increased. Irradiation at 1.6 kGy did not significantly affect color, soluble solid content, titratable acidity, or apple aroma intensity. The only negative effect of irradiation on apple slices appeared to be a loss of firmness. Our results suggest that CaA, used as an antibrowning agent, protected *L. monocytogenes* from radiation both in solution and on apple slices, but radiation at doses sufficient to inactivate 5-log of the bacterium did not significantly influence product quality attributes except for the loss in firmness.

Keywords: irradiation, fresh-cut apple, calcium ascorbate, *Listeria monocytogenes*, quality

Introduction

Production of fresh-cut produce, particularly the fresh-cut fruit segment, has increased rapidly in recent years (IFPA 2004). To prolong shelf life and enhance safety, fresh-cut produce including apple slices must be stored at refrigerated temperatures, often using modified atmosphere packaging (MAP). However, psychrotrophic bacteria such as *Listeria monocytogenes* can grow at refrigerated temperatures even under modified atmosphere (Jacxsens and others 1999). There was at least 1 recall associated with fresh-cut apples due to possible contamination with *L. monocytogenes* (USFDA 2001). *L. monocytogenes* is a common contaminant of ready-to-eat food products (Ryser and Marth 1999). The United States government established a "zero-tolerance" policy for *L. monocytogenes* on ready-to-eat foods (USDA-FSIS/DHHS-FDA 1992) because of the high mortality rate associated with listeriosis, especially for immune-compromised segment of the population.

Processing of fresh-cut apples induces mechanical damage of the fruit and exposes apple tissues to air, resulting in the development of undesirable tissue browning due to enzymatic and nonenzymatic reactions. The fresh-cut industry currently uses antibrowning agents such as calcium ascorbate (CaA) to prevent discoloration (Chen and others 1999; Karaibrahimoglu and others 2004). However, the antibrowning agent can become contaminated with *L. monocytogenes*, and washing of apple slices with the contaminated solutions can result in the transfer of pathogens to the product (USFDA 2001; Karaibrahimoglu and others 2004). Innovative food safety technologies are needed to inactivate foodborne pathogens in both antibrowning solutions and on the products.

Ionizing radiation effectively inactivates foodborne pathogens in various fruits and vegetables (Prakash and others 2000; Niemira 2003; Niemira and others 2003). Radiation resistance of a pathogen is commonly described using the D_{10} value, which is the radiation dose that inactivates 90% of a given bacterial population. The effectiveness of ionizing radiation depends on many factors. The radiation resistance of pathogens varies not only by the type of pathogens, but also by the type of product on which the pathogens are inoculated. The variation in radiation resistance of pathogens among different types of products can be affected by the presence of endogenous and exogenous antioxidants. Sommers and others (2002) showed that, in solutions, the antioxidant sodium erythorbate (an isomer of ascorbic acid) increased the radiation resistance of *L. monocytogenes*. However, when the antioxidant was used on inoculated meat samples, there was no effect on the radiation resistance. CaA, used by industry at concentrations as high as 9%, is a strong antioxidant. It is unclear whether CaA can influence radiation resistance of *L. monocytogenes* in solutions and on apple slices.

Although irradiation can inactivate foodborne pathogens, it may impact product quality if high doses are used. The major quality concerns include softening, tissue injury, and changes in flavor. Gunes and others (2001) demonstrated that irradiation doses above 0.34 kGy reduced the firmness of minimally processed apples. The Natl. Advisory Committee on Microbiological Criteria for Foods

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(1999) recommends a 5-log reduction of pathogens using single or hurdle food safety interventions. It is unclear whether quality attributes and the antibrowning effect of CaA during storage are altered by a 5-D_{10} radiation dose. Fan and others (2005) showed that irradiation at doses up to 1.0 kGy reduced microflora populations on apple slices but did not influence the antibrowning activity of CaA.

The objectives of this study were to evaluate the impact of CaA on the radiation resistance of *L. monocytogenes* inoculated into solutions and on apple slices and to study the effect of a 5-D_{10} irradiation dose on quality of apple slices.

Materials and Methods

Fruit source and assessment of fruit maturity

'Gala' apple fruit (*Malus* × *domestica* Borkh.) harvested at commercial maturity were stored at 1.5% O_2 and 0.5% CO_2 (balance N_2) at 1.5 °C for 6.5 mo before being used in this study. The fruit were selected so that the weight of fruits was 182 ± 14 g, corresponding to size 100 (100 fruit/bushel). Firmness, titratable acidity, soluble solids content, and pH of whole fruits were measured on the day of processing.

Determination of radiation resistance of *L. monocytogenes*

Strains. Three *L. monocytogenes* strains (H7762 serotype 4b, H7764 serotype 4b, and F4249 serotype 1/2 a) were obtained from the Centers for Disease Control and Prevention (Atlanta, Ga., U.S.A.). The strains were propagated on Tryptic Soy Agar (TSA) (BBL/Difco, Sparks, Md., U.S.A.) at 37 °C and maintained at 0 °C to 2 °C until they were used.

Bacterial cultures. Each *L. monocytogenes* strain was cultured independently in 100 mL of Tryptic Soy Broth (BBL/Difco, Sparks, Md., U.S.A.) in a baffled 500-mL Erlenmeyer culture flask at 37 °C at 150 rpm for 18 h. The method of cell propagation and inoculation was similar to the method described by Sommers and others (2002).

Inoculation of *L. monocytogenes* in CaA solution. After centrifugation, the *L. monocytogenes* cell pellets were resuspended and combined in 50 mL Butterfield's Phosphate Buffer (BPB) (Applied Research Inst., Newtown, Conn., U.S.A.). A 21% CaA (Spectrum Chemical Mfg., Corp., New Brunswick, N.J., U.S.A.) solution was prepared with distilled water and filter sterilized using a Nalgene 0.2 μm Sterilization Filter Unit (Nalgene Brand Products, Rochester, N.Y., U.S.A.). The inocula in 0%, 3.5%, and 7% CaA solutions were prepared by diluting the inoculum in BPB using 21% stock CaA solution. The samples (7.5 mL in 16 × 150 mm test tubes) were stored at 2 °C until irradiated, typically, for 30 to 60 min. After irradiation at 0 (control), 0.6, 1.2, 1.8, 2.4, and 3.0 kGy, the samples were returned to 2 °C storage until enumeration, about 30 to 60 min later.

Inoculation of *L. monocytogenes* in CaA solutions on apple slices. For the apple study, the same method as in the solution study was used to prepare a *L. monocytogenes* inoculum in 0%, 3.5%, or 7% CaA BPB solutions. All cutting boards, apple slicers, utensils, and holding containers were sanitized with 300-ppm chlorine. The apples were surface sanitized by submerging the apples in 300-ppm chlorine for 2 min. The areas in the calyx and stem end were cleaned using a sterile brush. The apples were cut into 8 slices with the core removed using apple slicers (OXO Good Grips 32681, OXO Intl., New York, N.Y., U.S.A.). The apples were sliced into 1 of the 3 dipping solutions contained in sterile glass dishes. Eighteen slices were dipped into 1 L inoculum/CaA solution. The population of the bacterium in the solution was in the range of 10^7 colony-forming units (CFU)/mL. After 2 to 3 min of treatment, the slices were drained, and 3 slices were placed into Model 400 Stomacher Bags (Seward Stomacher Lab System, London, U.K.). The samples were stored at 2 °C until irradiated, typically, for 30 to 60 min. The bags were left unsealed and then irradiated at 0 (control), 0.2, 0.4, 0.6, 0.8, and 1.0 kGy at 2 °C. After irradiation, the apple slice samples were returned to 2 °C storage for about 30 to 60 min until enumeration.

Enumeration. After the samples (solutions and apple slices) were irradiated, they were assayed for cell survival counts by standard pour plate procedures. For apple slice samples, 90 mL of sterile BPB was added to the stomacher bags containing apple slices. The sample bags were agitated by hand for 1 min to obtain a surface wash of the sample. The solutions and the washes from the apple slices were serially diluted in BPB using 10-fold dilutions, up to 10^6. Then, 1 mL of diluted sample was pour-plated in triplicate using 15 to 20 mL TSA into petri dishes (15 mm × 100 mm). The plates were inverted and incubated for 48 h at 37 °C before enumeration of colonies. Plates were counted with an automated plate counter (Accu Count 1000, Biologics, Gainsville, Va., U.S.A.). The bacterial population was expressed as CFU/g of apple slice or CFU/mL of solution. The experiments (solution and apple slices) were repeated independently 3 times. The data for each replicate were normalized against the control and plotted as the log reduction versus radiation doses. The D_{10} values were obtained by taking the negative reciprocal of the linear regression curves.

Quality study of apple slices

Apple slice preparation and CaA treatment. Processing of fresh-cut apples for the quality study was performed in an 8 °C clean processing room. All cutting boards, holding vessels, and then the fruit surfaces were sanitized with 300 ppm NaOCl (pH 9.2). Apple slicers were used to slice the apples into 8 equal pieces and to remove the core. The sliced apples were dropped into either water (0% CaA), 3.5% CaA, or 7% CaA solution for 2 to 3 min. Forty-eight slices were dipped into 2.5 L solution per replicate. The slices were then drained using plastic drainers and placed into multilayered polyolefin film bags (PD-900, Sealed Air Corp. Saddle Brook, N.J., U.S.A.) with O_2 and CO_2 transmission rates of 15.3 and 50 nmol/m²/s/kPa (3000 and 9800 mL/m²/24 h/atm) at 23 °C, respectively. The bags were sealed using an AIE-300 heat sealer (American Intl. Electric, Whittier, Calif., U.S.A.). The bag sizes were approximately 12 × 18 cm, and there were 8 slices (approximately 160 g) per bag. The packaged apple slices were either nonirradiated (0 kGy) or irradiated at 1.6 kGy at 4 °C. The dose was chosen based on the D_{10} values obtained from the inoculated slice experiment, so that a minimum of 5-D_{10} reduction of *L. monocytogenes* could be achieved with all apple slices, even those treated with 7% CaA. Titratable acidity, pH, and soluble solids contents and O_2 and CO_2 in the package headspace were measured as described earlier (Fan and others 2005) on the day of irradiation (0 d), and at 7 and 14 d of storage at 4 °C. Apple aroma intensity was evaluated by a consumer panel after 14 d of storage.

Sensory evaluation of apple aroma intensity. After 14 d of storage at 4 °C, the apple aroma intensity of slices was evaluated in the environmentally controlled booths at the sensory evaluation room of USDA's Eastern Regional Research Center (ERRC). The booths were illuminated with red light supplied by 4.75-inch dia primary red color filter (AF4RD, Genlyte Co., Fall River, Mass., U.S.A.) fitted on indoor halogen PAR 30 1100 lumens, 75 watts, narrow floodlight lamps (Osram Sylvania Products Inc., Winchester, Ky., U.S.A.). Each apple slice was cut into half, and each half was placed into a 2 oz (59.2 mL) polystyrene soufflé, sealed with PETE lids (Solo Cup Co., Urbana, Ill., U.S.A.), and equilibrated for 30 to 60 min at 23 °C before serving to panelists. Panelists consisted of ERRC employees. Each sample was given a 3-digit numerical code and order of pre-

sentation was randomized. In each session, panelists evaluated 6 samples, which were presented sequentially. Two sessions were conducted with 64 panelists in each session. Panelists were asked to open the containers, sniff the headspace of the containers, and rate the samples for apple aroma on a 0- to 10-line scale, anchored and labeled on both ends, where 0 represents no apple smell and 10 represents the strongest apple aroma. A comment section in the ballots was provided.

Irradiation and dosimetry

The samples were irradiated using a self-contained Cesium-137 gamma radiation source (Lockheed Georgia Co., Marietta, Ga., U.S.A.) containing 23 ^{137}Cs pencils placed in an annular array. To expose samples to radiation, samples in the stainless-steel cylindrical sample chamber (63.5 cm in height × 22.9 cm inner dia) were lowered into radiation source. The dose rate was 0.092 kGy/min. Irradiation, dosimetry, and control of temperature has been described by Fan and others (2005). Actual doses were within 5% of the target doses.

Experimental design and statistical analysis

The experimental design was a split-plot design with the whole plot factor being the CaA treatment and the subplots consisting of completely randomized design of the radiation doses and storage times. Three or 4 independent experiments were conducted to determine the radiation resistance of *L. monocytogenes*. For the quality study, 2 trials were performed. Within each trial, slices prepared from 2 batches of apples were subjected to each treatment (all possible combinations of CaA treatment and irradiation doses). The data from the 2 trials were combined to give 4 replicates. The data were analyzed with SAS version 7 (SAS Inst., Cary, N.C., U.S.A.). Effects of storage time, radiation dose, and CaA were analyzed using the Duncan's Multiple Range test of General Linear Models procedure. Only significant ($P < 0.05$) results are discussed unless stated otherwise.

Results and Discussion

Fruit composition

Firmness, titrateable acidity (TA), pH, and soluble solid content of the whole fruit used for the processing of fresh-cut apples were 1.8 ± 3.7 kg, 0.30 ± 0.04 mg malic acid equivalent per 100 mL juice, 4.0% ± 0.3% and 13.6% ± 1.2%, respectively.

Effect of CaA on radiation resistance of *L. monocytogenes* in solutions

The population of *L. monocytogenes* inoculated into various concentrations of CaA solutions as a function of radiation dose is shown in Figure 1. As radiation dose increased, the population of *L. monocytogenes* decreased linearly. The R^2 values for 0%, 3.5%, and 7.0% CaA solutions were 0.95, 0.83, and 0.89, respectively. The D_{10}-value of *L. monocytogenes*, calculated by averaging the 3 to 4 individual trials, were 0.32, 0.61, and 0.58 kGy in 0, 3.5%, and 7% CaA solutions, respectively (Figure 2a). CaA in both concentrations significantly increased (about 90%) the radiation resistance of *L. monocytogenes*. There was no significant difference in the D_{10} values of the pathogen between 3.5% and 7.0% solutions. D_{10} values of 0.35 kGy (Sommers and others 2002) and 0.4 kGy (Farkas and others 1995) for *L. monocytogenes* have been reported in phosphate buffer. Our results show the D_{10} values in BPB without CaA was 0.32 kGy. The protective effect of antioxidants on *L. monocytogenes* in solutions has been reported by Sommers and others (2002). D_{10} values of *L. monocytogenes* in solutions increased if the concentration of sodium erythorbate was above 0.1%. At the sodium erythorbate level of 1%, the D_{10} values doubled compared with those without erythorbate. Our results suggest that CaA increased radiation resistance of *L. monocytogenes* and that much higher radiation doses (about 3.0 kGy) are needed to inactivate *L. monocytogenes* by 5-logs in the CaA solutions.

Effect of CaA on radiation resistance of *L. monocytogenes* on apple slices

The population of *L. monocytogenes* inoculated on apple slices

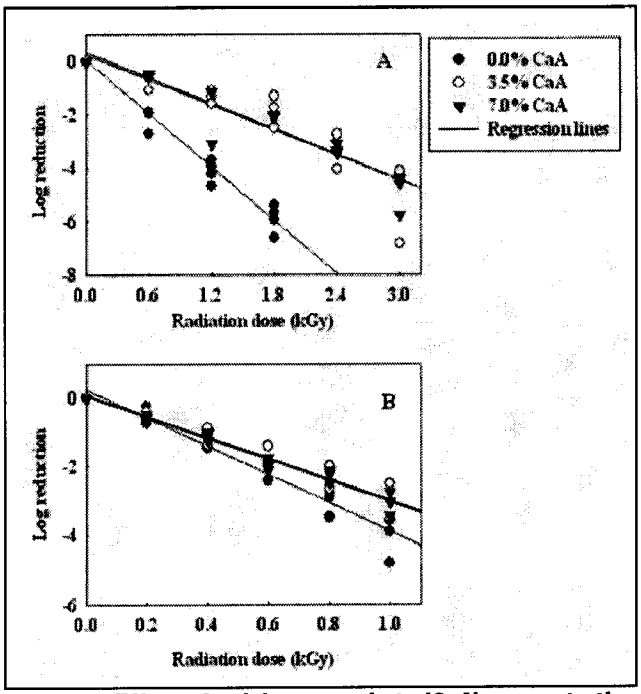

Figure 1—Effect of calcium ascorbate (CaA) concentration on radiation sensitivity of *Listeria monocytogenes* inoculated into solutions (a) and on apple slices (b)

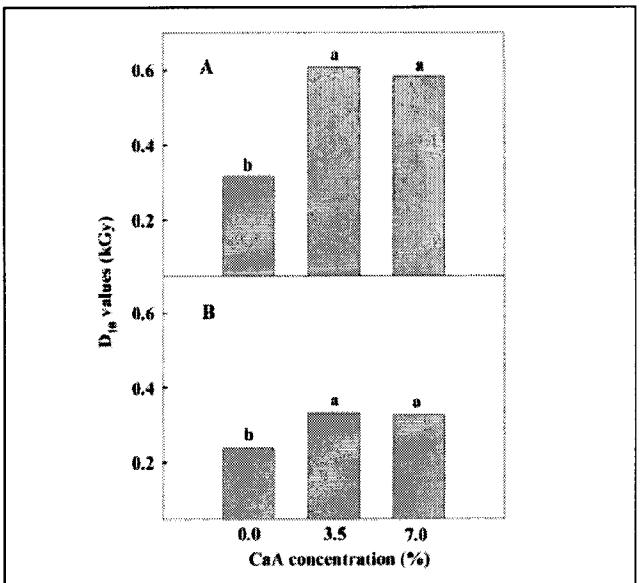

Figure 2—Effect of calcium ascorbate (CaA) concentration on radiation D_{10} values of *Listeria monocytogenes* in solutions (a) and on apple slices (b). Bars with same letters are not significantly different ($P > 0.05$, Duncan's multiple range test).

Table 1—Effects of irradiation dose and calcium ascorbate (CaA) on headspace O_2 and CO_2 levels in modified atmosphere packages of 'Gala' apple slices during storage at 4 °C[a]

	0 kGy			1.6 kGy		
Storage (day)	0% CaA	3.5% CaA	7.0% CaA	0% CaA	3.5% CaA	7.0% CaA
O_2 (kPa)						
0	20.1 ± 1.5abx	20.9 ± 0.5ax	20.6 ± 0.9abx	17.4 ± 2.2cx	17.3 ± 2.6cx	18.1 ± 0.9bcx
7	16.5 ± 3.0axy	12.2 ± 5.6ay	12.5 ± 5.6ay	9.3 ± 6.5ax	13.9 ± 5.4ax	11.9 ± 4.2ax
14	12.5 ± 4.1axy	8.7 ± 6.7ay	17.3 ± 0.6axy	11.1 ± 5.4ax	8.8 ± 6.8ax	13.3 ± 6.8ax
CO2 (kPa)						
0	—	—	—	1.9 ± 0.2ax	1.6 ± 0.0bx	—
7	2.9 ± 0.5ax	3.3 ± 0.9ax	3.6 ± 0.6ax	4.1 ± 0.8ay	3.7 ± 1.0ay	3.9 ± 0.4ax
14	3.4 ± 0.5ax	4.0 ± 1.0ax	3.1 ± 0.3ax	3.8 ± 0.5ay	3.5 ± 0.6ay	3.3 ± 1.0ax

[a]Values are means ± standard deviations, $n = 4$. Means in the same column (x-y) or in the same row (a-c) with same letters are not significantly different ($P > 0.05$). CO_2 values below detection limit (1.5 kPa) are indicated by '—'.

decreased linearly as dose increased (Figure 1b). The R^2 values for 0%, 3.5%, and 7.0% treatments were 0.95, 0.94, and 0.97, respectively. The D_{10} values for *L. monocytogenes* on the apple slices treated with 0%, 3.5%, and 7.0% CaA were 0.24, 0.32, and 0.32 kGy, respectively (Figure 2b). The 25% increase in D_{10} values due to CaA was significant ($P < 0.05$). Similar to the solution study, increasing CaA concentration from 3.5% to 7.0% did not further increase radiation resistance.

The D_{10} value of 0.24 kGy for *L. monocytogenes* inoculated on apple slices without CaA treatment is slightly higher than the values on lettuce (0.19 to 0.20 kGy) (Niemira 2003), celery (<0.20 kGy) (Prakash and others 2000), and endives (0.21 kGy) (Niemira and others 2003). The difference in the D_{10} values may be due to variations in strains of *L. monocytogenes*. In the present study, we used a cocktail of 3 strains of *L. monocytogenes*, whereas 1 or 2 strains were used in the previous studies.

Our results showed that CaA increased radiation resistance of *L. monocytogenes* inoculated in both solutions and on apples. Sommers and others (2002) found sodium erythorbate applied on beef bologna as a dip at concentration of 10% did not have any effect on D_{10} values (0.70 kGy) of *L. monocytogenes*. It is unclear what causes the difference in the 2 studies. Composition and surface characteristics of products may be factors. The protection effect of antioxidants on the pathogen was much stronger in solutions than on product. Water-soluble antioxidants (such as CaA and sodium erythorbate) may more easily infiltrate into cut apples then into bologna. Irradiation exerts its effect primarily through free radicals generated from radiolysis of water (Simic 1983). The free radicals attack bacterial cell membranes and DNA and either kill or damage microorganisms. CaA, an antioxidant, may scavenge free radicals generated from radiolysis of water, thereby reducing bacterial inactivation.

Our results clearly showed that CaA increased radiation resistance of *L. monocytogenes* inoculated on apple slices. Therefore, a higher dose is required to inactive *L. monocytogenes* on apples treated with CaA compared with those without CaA. The D_{10} values of *L. monocytogenes* inoculated on apple slices treated with either 3.5% or 7% CaA had D_{10} values of 0.32 kGy. To achieve a 5-log reduction of *L. monocytogenes* on apple slices, 1.6 kGy would be required. To investigate whether the 5-D_{10} dose (1.6 kGy) influences the quality of apple slices, we prepared apple slices without bacterial inoculation. The apple slices were then packaged in MAP followed by irradiation at 1.6 kGy and storage for 14 d at 4 °C.

Headspace atmosphere in the packages

Changes in O_2 and CO_2 levels in the packages during storage are presented in Table 1. Immediately after irradiation (day 0), the O_2 levels in the irradiated packages were 17.4, 17.3, and 18.1 kPa (1 kPa ≈ 1%) for 0%, 3.5%, and 7.0% CaA treated apples, respectively. The levels were significantly lower than those in corresponding nonirradiated packages, which were 20.1, 20.9, and 20.6 kPa, respectively. The CO_2 levels in the irradiated packages were 1.9, 1.7 kPa and undetectable (detection limit 1.5 kPa) for 0%, 3.5%, and 7.0% CaA treatments, respectively, whereas the CO_2 levels in all nonirradiated samples were below the detection limit. It appears that irradiation decreased O_2 levels and increased CO_2 levels at day 0. The increase in CO_2 and reduction in O_2 in the packages are presumably due to respiration of apple slices. Our results suggest that irradiation increased the respiration rate of apple slices. Earlier studies have shown that irradiation increased respiration rate in fresh-cut apples (Gunes and others 2000). During storage, the O_2 levels of all packages decreased to levels of 9.3 to 16.5 kPa at day 7 and 8.7 to 17.3 kPa at day 14, and CO_2 levels increased to 2.9 to 4.1 kPa at day 7 and 3.1 to 4.0 kPa at day 14. However, there was no significant ($P > 0.05$) difference in the O_2 and CO_2 levels between irradiated and nonirradiated packages either at day 7 or day 14. This suggests that the effect of radiation on respiration rate did not substantially alter gas composition in MAP during storage. Our results also suggest that the O_2 levels in the packages were much higher than the optimum levels for fresh-cut apples, which are presumably in the range of 1 to 2 kPa O_2 (Gorny 1997). The high O_2 level may be due to the low respiration rate of fruits after long-term controlled atmosphere cold storage and low storage temperature (4 °C) of fresh-cut apples.

Quality attributes

CaA treatment, irradiation, and storage time affected firmness (Table 2). At day 0, the nonirradiated apple slices treated with 3.5% CaA had higher firmness than the nonirradiated controls (0% CaA). Irradiated samples without CaA treatment and those treated with 3.5% CaA had lower firmness than corresponding nonirradiated samples. There was no statistical ($P > 0.05$) difference in the firmness of the 2 7% CaA-treated apple slices, suggesting that CaA at the higher concentration reduced the firmness loss due to irradiation. During storage, firmness of all samples did not change except that there was a decrease in nonirradiated samples treated with 3.5% CaA and irradiated samples treated with 7% CaA. At day 7 and day 14, firmness of irradiated samples at 3.5% and 7.0% CaA was lower than corresponding nonirradiated samples, demonstrating irradiation-induced losses in firmness occurred in CaA-treated apples during storage. It should be noted that on any given day of storage, the firmness of irradiated samples treated with 3.5% and 7.0% CaA was not significantly different than that of the nonirradiated samples that were not treated with CaA, suggesting that the combination of irradiation and CaA can achieve a similar quality attribute as those without irradiation and CaA. Irradiation reduced

Table 2—Effects of irradiation dose and calcium ascorbate (CaA) on firmness, titratable acidity (TA), pH, and soluble solids content of 'Gala' apple slices in modified atmosphere packages during storage at 4 °C[a]

	0 kGy			1.6 kGy		
Storage (day)	0% CaA	3.5% CaA	7.0% CaA	0% CaA	3.5% CaA	7.0% CaA
Firmness (kg)						
0	2.01 ± 0.37bcx	2.54 ± 0.39ax	2.30 ± 0.39abx	1.68 ± 0.43dx	1.79 ± 0.35dcx	2.13 ± 0.53bx
7	1.91 ± 0.45bcx	2.12 ± 0.53aby	2.30 ± 0.49ax	1.66 ± 0.37cx	1.65 ± 0.36cx	1.83 ± 0.24bcy
14	1.93 ± 0.42abx	2.23 ± 0.35ay	2.04 ± 0.58ax	1.49 ± 0.40cx	1.67 ± 0.35bcx	1.68 ± 0.35bcy
TA (mg malic acid equivalent /100 mL juice)						
0	0.29 ± 0.02abx	0.30 ± 0.03ax	0.30 ± 0.02ax	0.26 ± 0.02bx	0.29 ± 0.01ax	0.30 ± 0.01ax
7	0.28 ± 0.03ax	0.26 ± 0.02ay	0.29 ± 0.02ax	0.27 ± 0.01ax	0.27 ± 0.04axy	0.28 ± 0.04ax
14	0.24 ± 0.02ay	0.27 ± 0.01ay	0.26 ± 0.01ay	0.24 ± 0.03ax	0.25 ± 0.01ay	0.27 ± 0.02ax
pH						
0	4.0 ± 0.0bx	4.0 ± 0.1bx	4.2 ± 0.1ax	4.0 ± 0.1bx	4.0 ± 0.0bx	4.2 ± 0.1ax
7	4.0 ± 0.1bx	4.0 ± 0.0bx	4.3 ± 0.3ax	4.0 ± 0.0bx	4.0 ± 0.0bx	4.3 ± 0.2ax
14	4.0 ± 0.0bx	4.0 ± 0.0bx	4.2 ± 0.1ax	4.0 ± 0.1bx	4.0 ± 0.0bx	4.2 ± 0.2ax
Soluble solids content (%)						
0	12.8 ± 0.2bx	13.4 ± 0.4abx	13.5 ± 0.5abx	12.9 ± 0.4bx	13.3 ± 0.5abx	13.8 ± 0.8ax
7	12.7 ± 0.7ax	13.6 ± 0.2ax	13.5 ± 0.7ax	12.9 ± 0.7ax	13.2 ± 0.3ax	13.7 ± 1.1ax
14	12.1 ± 0.6bx	13.4 ± 0.2ax	12.4 ± 0.4abx	12.4 ± 0.4abx	12.9 ± 0.6abx	13.2 ± 1.1ax

[a]Values are means ± standard deviations, $n = 4$. Means in the same column (x-y) or in the same row (a-d) with same letters are not significantly different ($P > 0.05$).

Table 3—Effects of irradiation dose and calcium ascorbate (CaA) on instrumental color parameters of 'Gala' apple slices in modified atmosphere packages during storage at 4 °C[a]

	0 kGy			1.6 kGy		
Storage (day)	0% CaA	3.5% CaA	7.0% CaA	0% CaA	3.5% CaA	7.0% CaA
L*						
0	76.8 ± 2.6bx	79.8 ± 2.3ax	79.8 ± 1.8ax	76.5 ± 2.0bx	79.8 ± 2.0ax	79.3 ± 1.7ax
7	74.2 ± 2.2by	78.3 ± 2.2ay	77.7 ± 1.6az	74.4 ± 2.2by	78.4 ± 1.5ax	77.8 ± 1.2ay
14	74.4 ± 2.4cy	79.6 ± 1.5ax	78.8 ± 1.1by	75.1 ± 1.7cy	78.8 ± 1.3bx	78.4 ± 1.1by
Hue						
0	84.8 ± 2.0cx	90.4 ± 1.5ax	88.8 ± 3.2by	84.2 ± 2.3cx	89.3 ± 2.0abx	88.6 ± 2.4bx
7	83.6 ± 2.8dxy	90.9 ± 2.6ax	90.1 ± 3.3abxy	83.6 ± 2.2dx	89.5 ± 2.2bcx	88.4 ± 3.0cx
14	82.7 ± 2.8cy	91.1 ± 1.9ax	91.2 ± 4.0ax	83.6 ± 2.2cx	88.8 ± 2.0bx	88.6 ± 2.6bx
Chroma						
0	20.2 ± 2.0ax	15.0 ± 1.5cx	16.6 ± 2.3by	20.9 ± 2.5ax	15.0 ± 1.6cx	16.1 ± 1.9bxy
7	19.7 ± 2.2ax	14.6 ± 1.9cx	16.3 ± 2.4by	19.3 ± 1.9ay	14.4 ± 1.1cxy	15.2 ± 2.0cy
14	20.0 ± 2.1ax	14.7 ± 1.2dx	18.3 ± 3.1bx	19.7 ± 2.0axy	14.3 ± 1.5dy	16.3 ± 2.1cx

[a]Values are means ± standard deviations, $n = 4$. Means in the same column (x-z) or in the same row (a-d) with same letters are not significantly different ($P > 0.05$).

the firmness of apple slices, similar to earlier results on whole apples (Fan and Mattheis 2001) or apple slices (Gunes and others 2001; Fan and others 2005). Gunes and others (2001) found that irradiation at doses above 0.34 kGy reduced firmness, and the loss in firmness was correlated positively with water-soluble pectin and negatively with oxalate-soluble pectin content. Ca inhibits stress-induced senescence by maintaining membrane integrity (Nue and others 1986). The reduced rate of firmness loss during storage in apple slices treated with CaA may also be due to the role of calcium in cell membranes (Kovacs and others 1988) and the antioxidant activity of ascorbate. Antioxidants can scavenger irradiation-induced free radicals and therefore reduce the effect of irradiation on quality.

Titratable acidity of irradiated apple slices treated with all CaA levels was always similar with that of corresponding nonirradiated samples (Table 2). At day 0, titratable acidity of irradiated apple slices treated with 3.5% and 7.0% CaA was higher than those without CaA, but the difference disappeared during storage. During storage, titratable acidity decreased in all samples, but the decreases were not always statistically significant.

The pH of apple slices was not affected by irradiation or storage (Table 2). But apples treated with 7.0% CaA had higher pH than those treated with 3.5% CaA or those without CaA, regardless of irradiation and storage. The higher pH may be due to buffering by CaA solutions.

Soluble solids contents of irradiated samples treated at all CaA levels was similar to the corresponding nonirradiated ones at any day of storage (Table 2). Storage generally had no significant effect on soluble solids contents. Apple slices treated with CaA tended to have higher soluble solids contents, although not always significantly, than those without CaA. Soluble solids contents is highly correlated with sugar content of apples; however, other soluble matters may interfere with the measurement. It is possible that CaA added onto the sample may penetrate into apple tissues and increase the soluble solids contents reading.

The L^* values (lightness) and the hue angles of apples slices treated with CaA were higher than those without CaA, regardless of irradiation or storage duration (Table 3). The results indicate that the CaA treatment was effective in preventing darkening and browning of samples, and irradiation did not interfere with the CaA effects.

Chroma values of apple slices treated with CaA were lower than those without CaA, regardless of irradiation and storage duration, indicative of the absence of browning. Apple slices treated with

3.5% CaA always had the lowest chroma values regardless of irradiation. It is unclear why chroma values were the lowest at 3.5% CaA. CaA prevents tissue browning of apple slices, but ascorbic acid itself may be oxidized during storage, resulting in formation of browning pigments on apple slices. Chroma describes the saturation of a color. CaA treatment reduced chroma values of both irradiated and nonirradiated samples. These changes in chroma were mainly due to decreases in b^* values (blueness-yellow chromatism), indicating the CaA-treated samples became less yellow, and samples treated with 3.5% CaA generally had brighter and more vivid color than those treated with 7.0% CaA.

Irradiation had no effect on the intensity of apple aroma (Figure 3). The intensity however, decreased with increasing CaA concentration in both irradiated and nonirradiated samples. Calcium infiltration can reduce respiration and ethylene production and decrease the quantity of total aroma compounds of apple fruits (Conway and Sams 1987; Song and Bangerth 1993). In addition, CaA may mask the natural aroma of apple. Magee and others (2004) have found that some panelists can detect a change in the flavor of diced tomatoes after calcium dips. The apple aroma intensity was not affected by the gender of the panelists (data not shown). However, the age of the panelists had significant effects (Figure 4). The scores of apple aroma intensity decreased with increasing panelist age. Those older than 70 y gave much lower ratings of apple aroma intensity than the rest of the age groups. Panelists between ages 21 and 30 y gave the highest ratings.

After 14 d of storage at 4 °C, 3 of 96 slices treated with 3.5% CaA and 1.6 kGy radiation developed brown spots in the flesh that appeared near the calyx end. The brown spots were similar to internal CO_2 injury as evidenced by brown necrotic cortex tissue and were fairly well defined. No browning spots were found in slices of other treatments.

Antioxidants can affect radiation resistance of bacteria and radiation-induced changes in plant tissues. Sato and others (1995) suggested that antioxidants in plant tissues acting as radical scavengers protected the vacuolar membrane from radiation damage. Sommers and others (2002) showed that radiation resistance of the bacterium *L. monocytogenes* in cooked bologna was not affected by exogenous application of sodium erythorbate (an antioxidant) although sodium erythorbate increased radiation resistance of the bacterium in solutions. Niemira (2001) found that the radiation sensitivity of *Salmonella* in different types of orange juice was not affected by the composition of juices. Chiasson and others (2004) found that addition of ascorbic acid (0.5%) into ground beef increased radiation resistance of both *Escherichia coli* and *Salmonella Typhi*. Fan and Sokorai (2005) found that large differences in the endogenous antioxidant and phenolics content among fresh-cut vegetables did not always make a difference in their radiation sensitivity as measured by electrolyte leakage. The results of the present study suggest that CaA at 3.5% and 7% increased radiation resistance of *L. monocytogenes* in both solutions and on apple slices. But the protective effects of CaA against radiation inactivation of *L. monocytogenes* were much more pronounced in solutions than on apple slices. CaA may be diluted when it is in contact with the surface of apple slices as apple slices exude water to the surface. Also, before CaA treatment, there are many antioxidants already present on the surface of apple slices, which may protect bacteria against radiation damage, as evidenced by the much lower D_{10} values of *L. monocytogenes* on apple slices than in solutions.

Our results suggest that irradiation at 1.6 kGy did not significantly affect pH, soluble solids contents, or titratable acidity, but reduced firmness of apple slices. Earlier studies showed that irradiation at 0.44 kGy reduced firmness and titratable acidity of whole apples (Fan and Mattheis 2001), while a linear decrease in firmness as a function of radiation dose was observed in apple slices (Gunes and others 2001; Fan and others 2005). Our results also demonstrate that pretreatment with CaA before irradiation reduced the loss in firmness due to irradiation. As a result, apple slices treated with CaA and irradiation always had similar firmness compared with the nonirradiated, non-CaA–treated controls. An earlier study (Gunes and others 2001) found calcium pretreatment only reduced the irradiation-induced softening of very thin (3- to 4-mm thick) apple slices. CaA at 3.5% and 7% inhibited tissue browning of both irradiated and nonirradiated apple slices, suggesting that the antibrowning ability of CaA during storage was not altered by irradiation. It should be pointed out that the fruits used in this study had been stored for 6.5 mo in controlled atmosphere storage. After controlled atmosphere storage, fruits generally have low metabolic activity and may be less responsive to irradiation.

Although CaA treatment reduced irradiation-induced firmness loss and eliminated tissue browning during storage, it also reduced the intensity of apple aroma. Major volatile compounds that contribute to apple aroma are esters, alcohols, aldehydes, and ketones. Research is needed to study the impact of CaA on volatile aroma compounds of fresh-cut apples during storage and to investigate whether the low apple aroma intensity is due to calcium or the antioxidant (ascorbic acid) property of CaA.

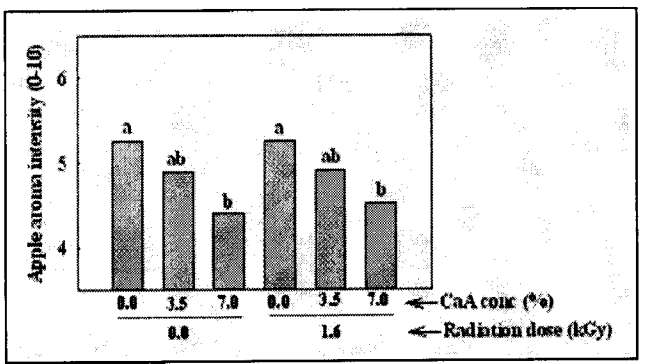

Figure 3—Effect of calcium ascorbate (CaA) and irradiation on apple aroma intensity (0 to 10) of 'Gala' apple slices stored for 14 d at 4 °C. Bars with same letters are not significantly different ($P > 0.05$, Duncan's multiple range test).

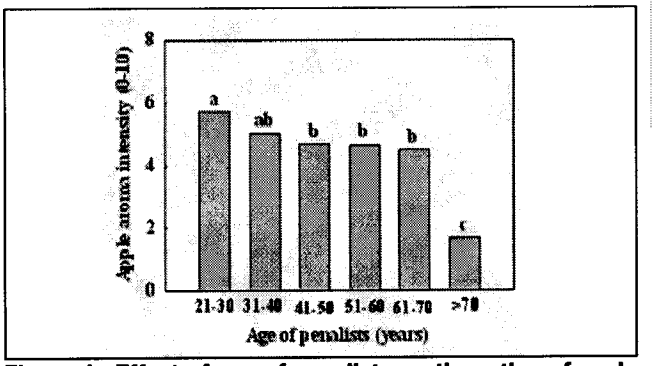

Figure 4—Effect of age of panelists on the rating of apple aroma intensity of 'Gala' apples slices stored for 14 d at 4 °C. Bars with same letters are not significantly different ($P > 0.05$, Duncan's multiple range test).

Conclusions

CaA, commonly used as an antibrowning agent for cut apples, increased radiation resistance of *L. monocytogenes* in solution and on apple slices, suggesting that a higher radiation dose is required to inactivate the pathogen in CaA-treated products. Radiation at 1.6 kGy, a dose that is sufficient to inactivate at least 99.999% of the pathogen on apple slices, did not affect color, aroma, soluble solids contents, titratable acidity, or pH of apple slices, but slightly reduced firmness. CaA reduced the loss of firmness due to irradiation, but it lowered the aroma intensity of apple slices regardless of irradiation. The combination of 3.5% CaA and irradiation effectively preserved the quality attributes of fresh-cut apple slices. Treatment with CaA and ionizing radiation resulted in microbiologically safe and good-quality fresh-cut apples.

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